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SYNTHESIS, CHARACTERIZATION AND BIOACTIVITY STUDY OF NOVEL BENZAMIDES AND THEIR COPPER AND COBALT COMPLEXES

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ABSTRACT

Metal complexes of four new benzamides, [N-(3'-nitrophenyl) (piperidin-1"-yl) methyl] benzamide, [N-(piperidin-1"-yl) (p-tolyl) methyl] benzamide, [N-(4'-chloro-phenyl) (piperidin-1"-yl) methyl] benzamide and [N-(4'-methoxy-phenyl) (piperidin-1"-yl] benzamide derived by the condensation of benzamide, piperidine and substituted benzaldehydes have been synthesized. The structural features of the newly synthesized compounds have been determined from their micro analytical, IR, NMR, UV-Vis, Mass, and ESR spectral and thermal analysis data. [N-(piperidin-1"-yl) (p-tolyl) methyl] benzamide ligand was analysed by single crystal X-ray diffraction. All the Cu (II) complexes exhibited octahedral and Co (II) complexes showed tetrahedral geometry. Magnetic susceptibility measurements and low conductance data provided evidence for the monomeric and non-electrolytic nature of the complexes, respectively. The electrochemical behavior of the complexes in acetonitrile at room temperature was studied. All synthesized compounds were evaluated for *in vitro* antibacterial activity against the bacterial strains: *Escherichia coli, Klebseilla pneumo*niae, *Bacillus subtilis*, *Staphylococcus aureus*, *Bacillus cereus*, *Proteus mirabilis* and *Pseudomonas aeruginosa.* The results were compared with standard antibiotic ampicillin and streptomycin. The copper complexes exhibited better activities than that of the free ligands and standard ampicillin against all bacteria. As compared to streptomycin, these were more active against bacteria *Bacillus cereus, ProteuS mirabilis* and *Pseudomonas aeruginosa*.

Key words: Benzamides, Antibacterial activity, Ampicillin, Streptomycin.

INTRODUCTION

The synthesis, structure and properties of amide compounds have been widely researched during the last several decades[1]. The compounds containing the amide moiety

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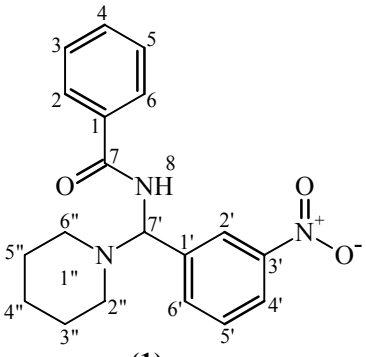
show a strong coordinating ability towards various transition metal ions and also exhibit diverse types of biological activities[2-4]. Metal complexes of benzamides as well as Mannich bases have been studied extensively in recent years due to the selectivity and sensitivity of the ligands towards various metal ions[5]. Mannich bases showed strong analgesic, sedative, antitussive and anticonvulsant activities[6]. Lot of work has been performed so far on isolation of solid complexes of different aromatic aldehydes or ketons and semicarbazones with transition metals[5]. There is currently a resurgence of interest in the biochemistry as well as the coordination chemistry of bivalent Cr, Co, Ni, Cu and Zn due to their biological importance[7]. Bio-activity of N-(1-morpholinobenzyl) semi carbazide and its transition metal complexes have been reported in literature[5]. Antibacterial properties of lanthanide (III) complexes of N-(pyrrolidinobenzyl) benzamide and transition metal complexes of N-(piperidinobenzyl) benzamide have been studied[8,9]. Earlier work reported that some drugs showed increased activity, when administered as metal complexes rather than as organic compounds[10]. Several antibiotics and therapeutic agents are known to produce their antibacterial effect after the formation of metal complexes[11].

Literature revealed that a further systematic study is required on this subject. Considering the bioactivity of various benzamide derivatives and their metal complexes, it was considered worth synthesizing and studying new benzamide derivatives, [N-(3'-nitrophenyl) (piperidin-1"-yl) methyl] benzamide, [N-(piperidin-1"-yl) (p-tolyl) methyl] benzamide, [N-(4'-chloro-phenyl) (piperidin-1"-yl) methyl] benzamide and [N-(4'-methoxy-phenyl) (piperidin-1"-yl] benzamide and their copper and cobalt complexes. A search through the literature reveals that no work has been carried out on these ligands and their metal complexes. We herein report the synthesis, characterization and bioactivity study of four new benzamides and their copper (II) and cobalt (II) complexes. In these ligand systems the coordination takes place via the nitrogen of the alicyclic ring and the oxygen of the carbonyl group. The proposed structures of the synthesized benzamides are given (Fig. 1).



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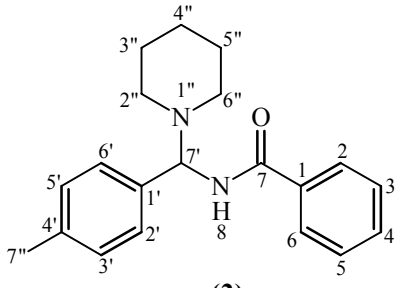

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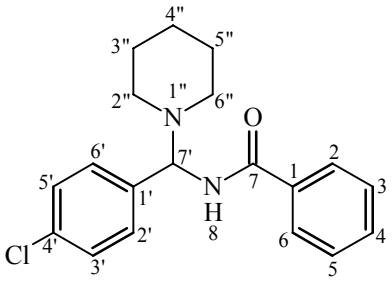

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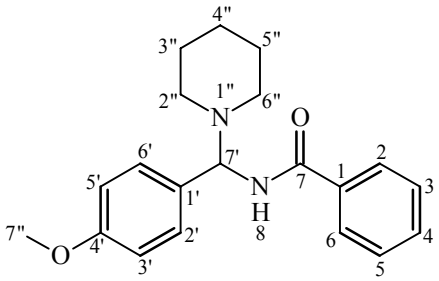

(4)

Fig. 1: Proposed structure of the benzamides

EXPERIMENTAL

Materials and methods

All the reagents used for the preparation of the ligand and the complexes were products of Merck, all AR grade. The IR spectra (4000-350 cm^{-1}) was recorded on Shimadzu FTIR-84005 spectrophotometer. NMR spectra were recorded on Bruker Armce III (400 MHz) machine. UV-VIS spectra were recorded on Shimadzu UV-1700 Thermaspec spectrophotometer. Mass spectra were recorded on Sciex API 3000 (ESI) spectrometer. The magnetic susceptibilities were measured on powdered samples using Guy balance. Shreetech/DTC 1SR thermal analyzer was used to record TG and DTA curves. ESR spectra of the samples in DMSO were obtained using a Varian E 112 X-band spectrometer, the field being calibrated with diphenyl picryl hydrazyl (DPPH) at RSIC, IIT, Mumbai.

Disc diffusion Agar technique was used to determine the antibacterial activities of the compounds against gram negative bacteria *Escherichia coli* (ATCC10536), *Klebseilla pneumoniae* (ATCC33495), *Proteus mirabilis* (ATCC12453) and *Pseudomonas aeruginosa (*ATCC10662*)* and gram positive bacteria *Bacillus subtilis* (ATCC11774), *Staphylococcus aureus* (ATCC1026) and *Bacillus cereus (*ATCC10876). Streptomycin and ampicillin were used as the standard.

Synthesis of mannich base

1.21 g (0.1 mol) benzamide was mixed with 0.98 mL (0.1 mol) of piperidine with constant stirring at room temperature. To this solution, ethanolic solution of either 1.51 g (0.1 mol) of m-nitro benzaldehyde or 1.4 g (0.1 mol) of p-chloro benzaldehyde or 1.19 mL of p-methyl benzaldehyde and 1.01 mL p-methoxy benzaldehyde was added drop wise under same conditions to prepare the corresponding benzamide derivatives. The resulting reaction

mixture was refluxed for 8 hr. The solution produced was kept at room temperature for four days. The crystalline product formed was washed with ethanol and recrystallized from acetone-hexane mixed solvent system[9].

Synthesis of metal complexes

For the synthesis of metal complexes, the ligand was dissolved in chloroform and mixed with an ethanolic solution of metal chloride, MCl_2 [M = Cu (II) and Co (II)] in 1 : 1 mole ratio. The resulting mixture was refluxed for 1 hr. Then the reaction mixture was kept overnight at room temperature. The supernatant liquid was removed and the solid product was washed with hexane and diethyl ether and dried in vacuo[9].

Procedure for antimicrobial activity

The *in vitro* antibacterial screening effects of the ligands and their complexes were tested against the above mentioned bacteria by disc diffusion method using Mueller-Hinton-Agar medium[12]. The stock solution was prepared by dissolving 10 mg of the compound in 1 mL DMF. The stock solution was serially diluted in order to find the MIC values. Sterile 6 mm diameter filter paper discs were impregnated with 40 µL of stock solution. The bacterial strains were inoculated on nutrients broth and incubated for 24 hrs at 37 ± 0.1°C. Adequate amount of Mueller-Hinton-Agar was dispensed into sterile plates and allowed to solidify under aseptic conditions. The count of the bacterial strains was adjusted to yield 1 x 108 mL^{-1}. The test organisms (100 µL) were inoculated with a sterile spreader on the surface of solid medium in place. The Agar plates inoculated with test organism were incubated for 1 hr before placing the stock solution impregnated paper discs on the plates. Following this the sterile discs impregnated with stock solutions were placed on Agar plates. The plates were incubated at 37 ± 0.1°C for 24 hrs. The inhibition zones were developed. The antibacterial activity results were calculated as a mean of three replicates. Streptomycin and ampicillin discs (10 mg mL^{-1}) were used as positive control.

RESULTS AND DISCUSSION

The benzamides were prepared as described in the experimental part, crystallized and dried in air and subjected to physical and spectral analysis. Analytical and physical data of benzamides and their complexes are presented (Table 1 and 2). The structure of benzamides has been characterized by using IR, NMR and mass spectral data. The coordination behaviour of benzamides towards transition metal ions was investigated via the IR spectra, molar conductance, magnetic moment, and thermal studies. The elemental analysis of the complexes show 1 : 1 (metal : ligand) stoichiometry for all the complexes.

The magnetic moment of the complexes were measured at room temperature. Both copper and cobalt complexes have paramagnetic character. The low molar conductance values of the complexes reveal their non-electrolytic nature.

NMR Spectra

^{1}H NMR of the ligands was recorded in $CDCl_3$ solution. ^{1}H NMR spectrum of the ligands indicates characteristic integration pattern of aromatic moieties at between δ 8.38-7.31. A doublet at δ 6.65-6.57 is observed due to amide nitrogen proton. A doublet is detected at δ 6.12-5.90 as a consequence of methine proton. Two merged broad peaks of piperidine ring are observed at δ 2.6-2.5 methylene protons. Multiplets are detected at δ 1.59 and δ 1.45 for methylene protons of piperidine ring. The δ shift values support the proposed structure of the ligands.

Table 1: Ligands and complexes prepared

Ligand/complex	Numbered in text as	Molecular formula	Yield (%)
L1	1	$C_{19}H_{21}O_3N_3$	74
L2	2	$C_{20}H_{24}ON_2$	79
L3	3	$C_{19}H_{21}O\ N_2Cl$	76
L4	4	$C_{20}H_{24}N_2O_2$	72
[L1CuCl.2 (H_2O)]	1a	$C_{19}H_{20}O_3N_3.CuCl.2H_2O$	57
[L2$CuCl_2$.2 (H_2O)]	2a	$C_{20}H_{24}ON_2.CuCl_2.2H_2O$	56
[L3$CuCl_2$.2 (H_2O)]	3a	$C_{19}H_{21}ON_2Cl.CuCl_2.2H_2O$	58
[L4$CuCl_2$.2 (H_2O)]	4a	$C_{20}H_{24}N_2O_2.CuCl_2.2H_2O$	50
[L1CoCl].2 (H_2O)	1b	$C_{19}H_{20}O_3N_3.CoCl.2H_2O$	50
[L2$CoCl_2$].2 (H_2O)	2b	$C_{20}H_{24}ON_2.CoCl_2.2H_2O$	56
[L3$CoCl_2$].2 (H_2O)	3b	$C_{19}H_{21}O\ N_2Cl.CoCl_2.2H_2O$	54
[L4$CoCl_2$].2 (H_2O)	4b	$C_{20}H_{24}N_2O_2.CoCl_2.2H_2O$	51

Table 2: Analytical and physical data

Product	Colour	MP (°C)	Analytical data % found (Cal)					M Wt.	μ_{eff} (BM)	Λ_M ($\Omega^{-1}cm^2$ mol^{-1})
			M	C	H	N	O			
1	White	175	-	-	-	-	-	339	-	-
2	White	150	-	-	-	-	-	308	-	-
3	White	148	-	-	-	-	-	328	-	-
4	White	140	-	-	-	-	-	324	-	-
1a	Green	200 (Dec.)	12.8 (13.2)	47.9 (48.1)	4.9 (5.2)	8.5 (8.8)	16.4 (16.8)	475	1.90	10.8
2a	Green	180 (Dec.)	13.0 (13.5)	49.8 (50.1)	5.1 (5.8)	5.6 (5.8)	9.9 (10.0)	479	1.89	10.5
3a	Green	175 (Dec.)	12.7 (13.0)	44.8 (45.6)	4.5 (5.0)	4.9 (5.6)	9.5 (9.6)	499	1.91	11.0
4a	Green	190 (Dec.)	12.2 (12.7)	48.0 (48.6)	5.1 (5.6)	5.0 (5.6)	12.1 (12.9)	493	1.88	10.8
1b	Blue	140 (Dec.)	12.9 (13.3)	48.3 (48.6)	5.0 (5.3)	8.7 (8.9)	16.8 (17.0)	469	4.52	4.2
2b	Blue	170 (Dec.)	11.8 (12.2)	49.9 (50.6)	6.0 (5.9)	5.5 (5.9)	10.0 (10.1)	474	4.45	3.8
3b	Blue	195 (Dec.)	11.1 (11.7)	45.2 (46.2)	4.8 (5.1)	5.0 (5.6)	9.5 (9.7)	494	4.50	3.2
4b	Blue	175 (Dec.)	11.1 (11.8)	48.8 (49.1)	5.2 (5.7)	5.01 (5.7)	12.7 (13.1)	488	4.6	3.0

X-ray crystallography of [N-(piperidin-1"-yl) (p-tolyl) methyl] benzamide (Compound 2)

Single crystals of compound 2, [N-(piperidin-1"-yl) (p-tolyl) methyl] benzamide, were developed by slow evaporation of ethanolic solution of the compound at room temperature. Molecular structure of the bioactive crystal was unambiguously determined by single crystal x-ray diffraction for the first time. Colourless crystal of approximate size 0.25 x 0.06 x 0.02 mm^3 was used for data collection on Bruker Smart Apex CCD diffractometer using Mo K_α radiation $C_{20}H_{24}N_2O$, M = .308.41. Crystals belong to

monoclinic, space group $P2_1/c$, a = 9.1178 (5) Å, *b* = 19.5119 (8) Å, c = 9.9911 (4) Å, β = 101.623 (4)°, V = 1741.02 (14) Å 3, *Z* = 4, D_c = 1.177 g/cc, 3058 reflections measured [I > 2σ (I)], R value, R^1 = 0.0497, wR^2 = 0.1171. X-ray analysis revealed the planar confirmation of the two benzene rings and chair conformation of piperidine ring. ORTEP diagram of compound 2 is shown in (Fig. 2).

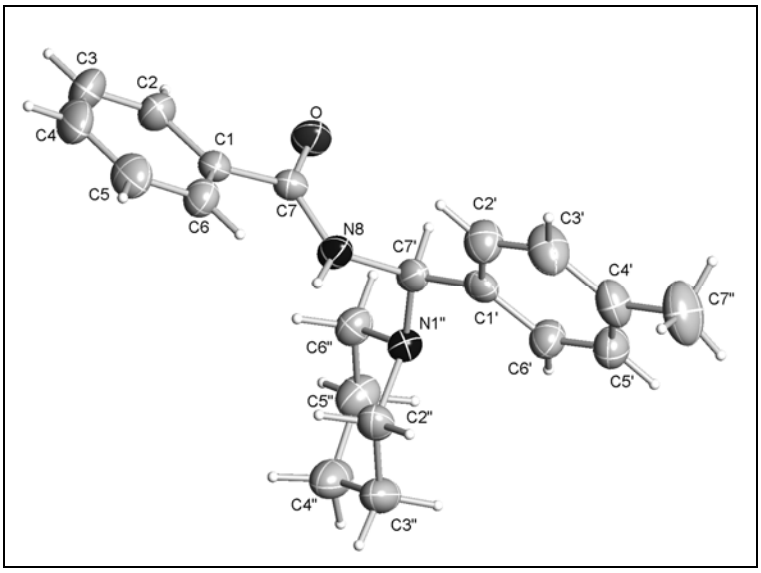

Fig. 2: ORTEP diagram of Compound 2, Ellipsoids drawn at 50% Probability

Spectral analysis

In order to study the binding mode of the ligand in the complexes, the IR spectra of the free ligand is compared with the spectra of the complexes (Table 3). In complex 1a and 1b peak observed at 3267 cm^{-1} for amide – NH in ligand (compound 1) is absent in the spectrum of complex, 1a and 1b. The disappearance of this band indicates the deprotonation of amide – NH group upon coordination. The amide ν C=O mode of ligand at 1643 cm^{-1} is found to shift to lower frequency in both the complex suggesting the coordination of carbonyl oxygen to the central metal ion. Bands seen in fingerprint region due to C-N-C stretching of piperidine ring also display a distinctly different nature in the spectra of complexes. Thus it can be concluded that compound 1 behaves as a tridentate ligand, binding through amide nitrogen with deprotonation, piperidine nitrogen and carbonyl oxygen. Such type of coordination is reported for Schiff base copper complexes[10]. In other complexes, 2a, 2b, 3a, 3b, 4a and 4b, the amide ν C=O mode of ligand is found to shift to lower frequency suggesting the coordination of carbonyl oxygen to the central metal ion.

Similarly the ν C-N-C of piperidine ring underwent a shift in frequency after complexation, indicating the coordination through nitrogen of piperidine entity present in the ligand. Thus, it can be concluded that the in these complexes the ligands behave as a bidentate ligand and coordination of metal ion occurs through the piperidine nitrogen and the carbonyl oxygen. All copper complexes (1a, 2a, 3a and 4a) exhibit a peak at 3348-3448 cm^{-1} along with a new peak at around 850 cm^{-1} characteristic for coordinated water molecule, which is confirmed by TG analysis. All Cobalt complexes (1b, 2b, 3b and 4b) show a peak at 3561-3234 cm^{-1} for water molecule. In the far-infrared region all metal complexes exhibit new bands around 554-536 cm^{-1} and 472-430 cm^{-1}, which are tentatively assigned for M-O and M-N bonds respectively[13,14]. In all the complexes, an additional band at 399-374 cm^{-1} is assigned to M-Cl stretching vibration[15].

Table 3: Important IR frequencies (cm^{-1}) of the ligands and their Complexes

Compd.	Peaks (ν cm^{-1})						
	-C=O	Amide-NH	ν C-N-C (Piperidine)	H_2O/OH	M-Cl	M-O	M-N
1	1643	3268	1205-1099	-	-	-	-
1a	1610	-	1165-1080	3348-3448 New 850	389	550	453
1b	1600	-	1163-1080	3537-3151	381	547	459
2	1640	3325	1269-1101	-	-	-	-
2a	1620	3348,3451	1161-1078	3348-3450 New 854	399	549	472
2b	1593	3450 - 3240	1163-1080	3554	393	547	430
3	1642	3320	1267-1097	-	-	-	-
3a	1610	3400-3300	1159-1078	3454	389	543	432
3b	1626	3538-3151	1168-1080	3151-3537	374	-	-
4	1636	3314	1250-1105	-	-	-	-
4a	1657	3348-3350	1163-1043	3350-3448 New 860	370	553	440
4b	1662	3360	1215, 1080	3360	392	551	450

Mass spectra

The mass spectra of the ligands and their complexes were recorded and their stoichiometric composition compared. Ligand **1** ($C_{19}H_{21}N_3O_3$: Mol. Wt.= 339) gives the molecular ion peak at m/z = 340 in (+)ve mode whereas its copper complex, 1a ($C_{19}H_{21}N_3O_3CuCl_2.H_2O$) shows the molecular ion peak at m/z = 531 in (-)ve mode, which is associated with one sodium ion and cobalt complex, 1b shows molecular ion peak at $[M + 1]^+$ *m/z* 468. The data indicates the molecular mass of complex to be 467 with molecular formula $C_{19}H_{20}O_3N_3CoCl.2H_2O$. Ligand **2** ($C_{20}H_{24}ON_2$, M.W 308) gives the molecular ion peak at m/z =309 in (+)ve mode and its complex, 2a shows molecular ion peak at m/z = 498, which is associated with one NH_4^+ ion. Its cobalt complex, 2b shows a molecular ion peak at $[M-1]^+$ *m/z* = 496, which is associated with one Na^+. The mass data denotes the molecular formula is to be $C_{20}H_{24}ON_2CoCl_2.2H_2O$ with molecular mass 474. Ligand **3** ($C_{19}H_{21}O\ N_2Cl$, MW 328) exhibited molecular ion peak at m/z = 329 in (+)ve mode and its Cu complex, 3a shows the molecular ion peak at m/z = 498. 3b exhibits molecular ion peak at $[M + 1]^+$ *m/z* 493. The mass data supports the molecular mass of complex, 3b to be 492 with molecular formula ($C_{19}H_{21}ON_2ClCoCl_2.2H_2O$). Similarly complexes 4a and 4b show molecular ion peak at $[M-1]^+$ *m/z* 513 which is an adduct of NH_4^+ ion and at $[M-1]^+$ *m/z* 487, respectively.

Electronic absorption spectra

The electronic absorption spectra of the ligand and its Cu and Co complexes were recorded at room temperature in DMF solution. The complexes 1a, 2a, 3a and 4a showed band at 14727 cm^{-1}, 14836 cm^{-1}, 14836 cm^{-1} and 14858 cm^{-1} which is assigned to $^2Eg \rightarrow {}^2T_{2g}$ transition. This confirms the octahedral geometry of the complexes[16]. The Co complexes 1b, 2b, 3b and 4b exhibited band at 14, 836 cm^{-1}, 14992 cm^{-1}, 14858 cm^{-1} and 14836 cm^{-1} assigned as $^4A_2 \rightarrow {}^4T_1$ transition. This supports the tetrahedral geometry of the complexes[17]. No peak was observed for the ligands in this region.

ESR Spectra

The ESR spectrum of metal complexes provides information about the stereo-chemistry and the site of metal ligand bonding which are important in studying the metal ion environment in complexes. It also helps to determine the magnetic interaction in the metal complexes. The X-band ESR spectra of all Cu (II) complexes, recorded in DMSO at room temperature (300 K) (Fig. 3) showed one intense absorption band in the high field and are isotropic due to the tumbling motion of the molecule. The g tensore values of Cu complexes can be used to derive the ground state. All the copper complexes exhibited the $g_{\|}$ in the

range of 2.10 to 2.24 and $g_{\perp}$ in the range of 2.01 to 2.04. These values indicate that $g_{\parallel} > g_{\perp} > 2.0023$, indicating that the copper site has a $d_{x^2-y^2}$ ground state characteristic of octahedral geometry[18]. For a Cu (II) complex, $g_{\parallel}$ is a parameter sensitive enough to indicate covalency. The $g_{\parallel}$ values for all the Cu (II) complexes are less than 2.3 is an indication of signifivcant covalent bonding in these complexes[16]. According to Hathaway the geometric parameter, which is measure of exchange interaction between the copper centers in the complex is calculated using the equation, $G = g_{\parallel}-2 / g_{\perp} -2$. If $G > 4$, the exchange interaction between the Cu (II) centers in the complex is negligible.[19] In all the above Cu (II) complexes, $G > 4$, indicating negligible exchange interaction of Cu-Cu in the complexes. The spin-orbit coupling constant, The spin orbit-coupling constant, λ value is calculated using the relation,

$$g_{av} = 1/3 [g_{\parallel} + 2 g_{\perp}) \text{ and } g_{av} = 2 (1- 2 \lambda/ 10 Dq)$$

λ value for all Cu (II) complexes are found to be less than the free Cu (II) ion (-832), which also supports covalent character. The in-plan σ-bonding covalency parameters, α^2 are related to $A_{\parallel}$, $g_{\parallel}$ and $g_{\perp}$ according to following equation[20].

$$\alpha^2 = - (A_{\parallel}/ 0.036) + (g_{\parallel} - 2.0023) + 3/7 (g_{\perp} - 2.0023) + 0.04$$

If the α^2 value is 0.5, it indicates complete covalent bonding, while the value of α^2 is 1.0 suggests complete ionic bonding. The α^2 values are found in the range of 0.3-0.5, indicating covalent character of M-L bond in the complex.

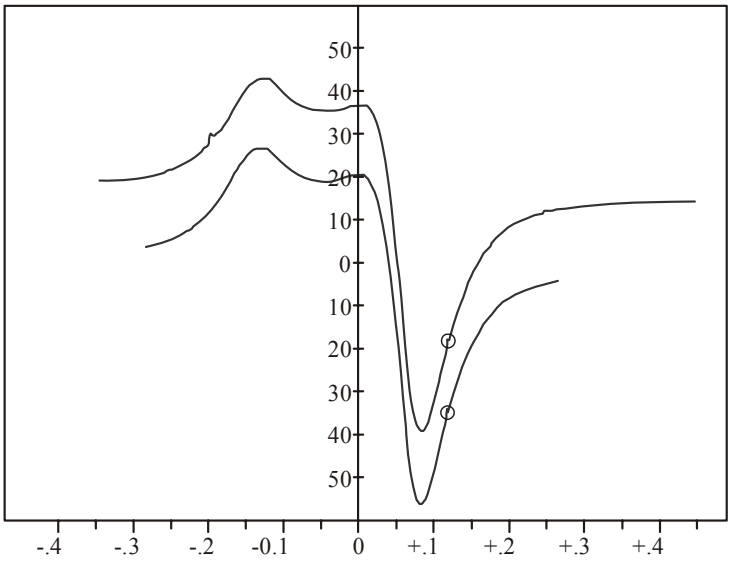

Fig. 3: ESR of Complex, 1a

Magnetic susceptibility measurements

The magnetic moment (μ_{eff}) of Cu (II) complexes, 1a, 2a, 3a and 4a is found in the range of 1.89-1.91 BM. This suggests an octahedral arrangement of ligand around the central metal ion. The magnetic moment of Co complexes 1b, 2b, 3b and 4b is in the range of 4.45-4.52 BM, which indicates the presence of three unpaired electrons. This supports the tetrahedral structure for the complexes[17].

Thermal studies

The thermogram of complex 1a shows 7.5% (cal. 7.08%) weight loss in the temperature range 164-200^{o}C and complex 2a exhibits 7.4% (cal.7.5%) weight loss in the temperature range 151-213^{o}C in first step corresponds for the removal of two water molecules. Similarly 3a exhibits 7.5% weight loss in the temperature range 144-292^{o}C and 4a shows 7.8% weight loss in the temperature range 151-277^{o}C. The loss of these weight fractions in this temperature range indicates the presence of two coordinated water molecules in all the Cu complexes.

The thermogram of cobalt complex, **1b** shows a 7.4% weight loss in the temperature range 121-148^{o}C and **2b** shows a 7.4 % weight loss in the temperature range 61-151^{o}C in first step decomposition. Similarly complexes, 3b and 4b exhibit 7.9% in 39-151^{o}C and 7.0% in the temperature range 40-121^{o}C for elimination of two water molecules. For all the co complexes (**1b, 2b, 3b and 4b**) the first step weight loss corresponds very well to the release of two water molecules and relatively low temperature of water loss indicates that this is lattice held.

Cyclic voltametry

The cyclic voltamogram of Cu complexes (0.001 M) in MeCN solution in 1.1-1.2V potential range were carried out to study the electrochemical properties of the complexes. The scan rates were varied from 50 to 250 mv/S.

The voltamogram of compound 1a (Fig. 4) shows a well defined redox process at 0.05 mv/S scan rate corresponding to the formation of Cu (II) / Cu (III) couple at anodic peak at Epa = 0.75V and associated cathodic peak at Epc = 0.400 V. This couple is found to be reversible with ΔEp = 0.350V. The ratio of anodic to cathodic peak currents (Ipa/Ipc=1) indicates that the process is simple one electron transfer process. The complex also shows a quasi-reversible peak in the negative region, at Epa = -0.05V with associated cathodic peak at Epc = - 0.95 V. The value of ΔEp is 0.9V reveals that the process can be

quasi-reversible. This quasi-reversible peak is characteristic of Cu (II) → Cu (I) couple. The compound exhibits same type of charge transfer for other scan rate. All Cu (II) complexes, 2a, 3a and 4a exhibit similar type of voltamogram. The Cyclic voltametric data are presented (Table 4).

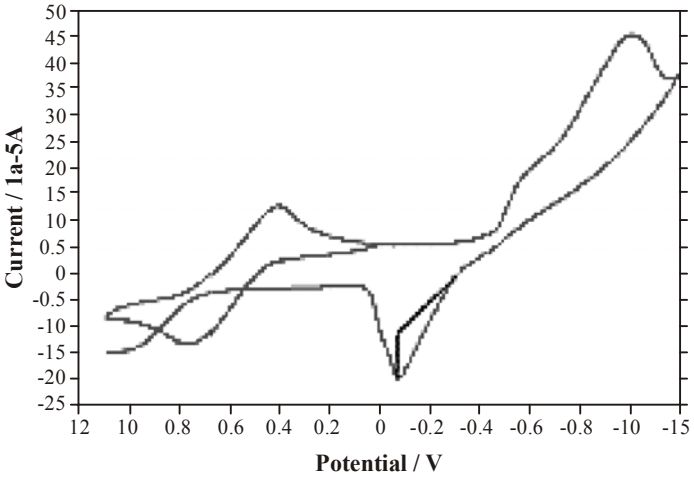

Fig. 4: Cyclic voltamogram of complex, 1a

Table 4: Cyclic voltametric data of copper complexes in MeCN solution for couple Cu (II) / Cu (III)

Complex	Epa (V)	Epc (V)	Ipa (amp)	Ipc (amp)	Ipa/Ipc
1a	0.750	0.400	-1.4×10^{-5}	1.3×10^{-5}	1.0
2a	0.800	0.450	-2.6×10^{-5}	1.6×10^{-5}	1.6
3a	0.800	0.500	-0.70×10^{-5}	0.45×10^{-5}	1.5
4a	0.750	0.400	-1.0×10^{-5}	0.9×10^{-5}	1.1

Scan rate 50 mv/S

The powder XRD diffraction patterns were recorded for Cu complex in the $2\theta = 3\text{-}80^{o}$ range. The patterns for all the four Cu complexes were found similar with a sharp peak indicating the crystalline nature of the complexes[21].

On the basis of spectral data, elemental analysis and thermal study the structure proposed for complexes are presented (Fig. 5, 6, 7 and 8).

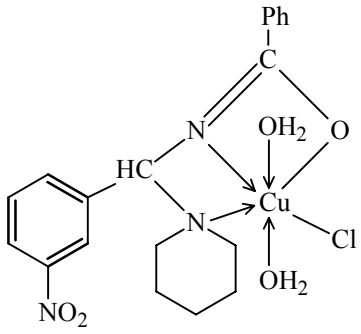

Fig. 5: Complex 1a

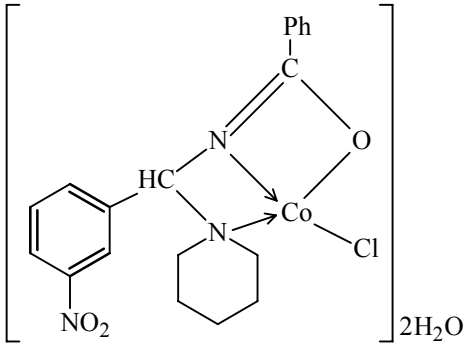

Fig.6: Complex 1b

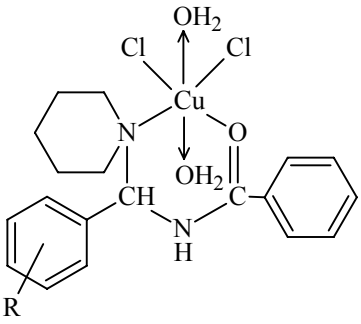

Fig.-7: Complexes 2a, 3a and 4a

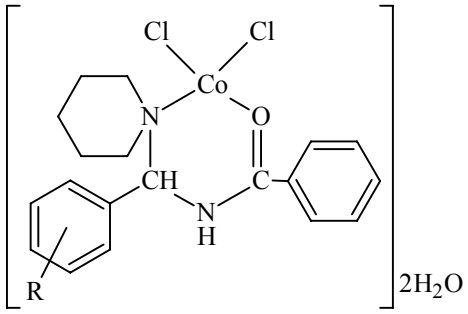

Fig. 8: Complexes 2b, 3b and 4b

(X= -CH_3, -Cl, -OCH_3)

Antibactrial activity

The *in vitro* antibacterial screening of the newly synthesized compounds were tested against *Escherichia coli*, *Klebseilla pneumonia, Proteus mirabilis* and *Pseudomonas aeruginosa (*ATCC10662*)* and *gram positive bacteria Bacillus subtilis, Staphylococcus aureus* and *Bacillus cereus*. The results of antibacterial activities of the compounds are presented (Table 5). The effect of synthesized compound on the growth of bacteria may be due to the interference of the compounds with protein synthesis of bacteria. The compounds may be able to attach sufficient portion of the bacterial ribosome, and in doing so, cause a misreading of messenger RNA. Thus faulty proteins are synthesized and are incapable of sustaining vital cell functions. In a short time the cell is killed[22]. As seen from the result, the ligands show higher activity than standard ampicillin and moderate activity as compared to streptomycin.

A comparative study of the ligand and its complexes indicates that the copper complexes exhibit higher antibacterial activity than the free ligand against all test organisms. All the copper complexes are found to be more active than standard antibiotic ampicillin against all tested organisms. These complexes show higher activities than the standard streptomycin against *Bacillus cereus*, *Proteus mirabilis* and *Pseudomonas aeruginosa* and show equal activity against *Escherichia coli*. The MIC is calculated for copper complex against *Bacillus cereus, Proteus mirabilis* and *Pseudomonas aeruginosa* and *Escherichia coli.* Cu complexes exhibit very significant activity at a MIC value of 25 µg/mL against *Bacillus cereus* and 12.5 µg/mL against *Proteus mirabilis* and *Pseudomonas aeruginosa* and *Escherichia coli.*

Cobalt complex are found to be almost inactive. The lower activity of cobalt complexes could be accounted on the basis of low lipid solubility. Hence, the metal ion cannot reach the desirable site of action of the cell wall to interfere with the normal cell activity.

The higher activity of copper complex on the growth of bacteria can be explained as the increase lipophilic character of metal complexes may be responsible for their more potent antimicrobial activity than the free ligand. The permeation of complexes through the lipid layer of the cell membranes deactivates diverse cellular enzymes, which play a vital role in various metabolic systems of these organism[1]. Such increased activity of complexes can also be explained on the basis of Overtone's concept of cell permeability[10], according to which the lipid membrane that surrounds the cell favours the passage of only the lipid soluble materials due to which liposolubility is an important factor, which controls the antibacterial activity. The higher activity of copper complex can also be explained on the basis of Tweedy's Chelation theory[10], according to which chelation increases the activity. On chelation, the polarity of copper ion will be reduced to a greater extend due to the overlap of the ligand orbital and partial sharing of the positive charge of the copper ion with donor groups. Further, it increases the delocalization of π-electrons over the whole chelate ring and enhances the lipophilicity of the complex. The increased lipophilicity enhances the penetration of the complexes into lipid membranes and blocking of the metal biding sites in the enzymes of microorganisms. The complex also disturbs the respiration process of the cell and thus blocks the synthesis of the proteins that restricts further growth of the organism. From the cyclic voltammetric behaviour, the redox properties of copper ion, may also contribute to their inherent toxicity. Redox cycling between Cu (II) and Cu (I) can catalyze the production of highly reactive hydroxyl radicals, which can subsequently damage lipids, proteins, DNA and other biomolecules. Although the exact mechanism is not understood

biochemically, it is quite possible that all of the above factors discussed may be responsible either individually or collectively for the observed antibacterial activity of complexes studied.

Table 5: Antibacterial activities

Compd.	Zone of inhibition* (mm)						
	Klebseilla Pneumoniae	***Bacillus subtilis***	***Staphylococcus aureus***	***Bacillus cereus***	***Proteus mirabilis***	***Pseudomonas aeruginosa***	***E. coli***
1	11	12	14	11	09	15	16
2	9	10	12	9	9	13	14
3	10	11	13	10	9	14	15
4	10	9	12	10	9	12	14
1a	13	11	16	20	25	28	29
2a	19	11	14	19	21	26	28
3a	13	12	16	20	25	28	29
4a	12	12	15	20	20	27	29
1b	NA	8	NA	NA	NA	NA	NA
2b	11	NA	NA	9	16	9	8
3b	NA	7	NA	NA	NA	NA	NA
4b	NA	NA	NA	NA	NA	8	8
Streptomycin	21	39	37	19	16	23	29
Ampicillin	08	08	07	08	07	10	10

*Zone of inhibition including the diameter of filter paper disc (5 mm)

**NA: No activity

CONCLUSION

The synthesized copper complexes of new benzamide ligands were found octahedral in structure. The cobalt complexes of these ligands were found tetrahedral. The ligands showed higher activity compared to ampicillin and moderate activity against bacteria *Klebseilla pneumoniae*, *Staphylococcus aureus*, *Bacillus cereus*, *Proteus mirabilis* and

Pseudomonas aeruginosa. Notably, the copper complexes were found more active than their parent compounds against all the tested bacteria. They exhibited higher activities against all bacterial strain than the standard ampicillin. These are superior than streptomycin against *Escherichia coli*, *Bacillus cereus*, *Proteus mirabilis* and *Pseudomonas aeruginosa*. The cobalt complex showed minimum activity. Activity increased with increasing concentration. In conclusion it can be point out that the activity of Cu (II) complexes as compared to both of the standard antibiotics is quite encouraging. They are found to be a superior antibacterial agent as compared to standard. These observations provide some predictions in order to design further antimicrobial active compounds.

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